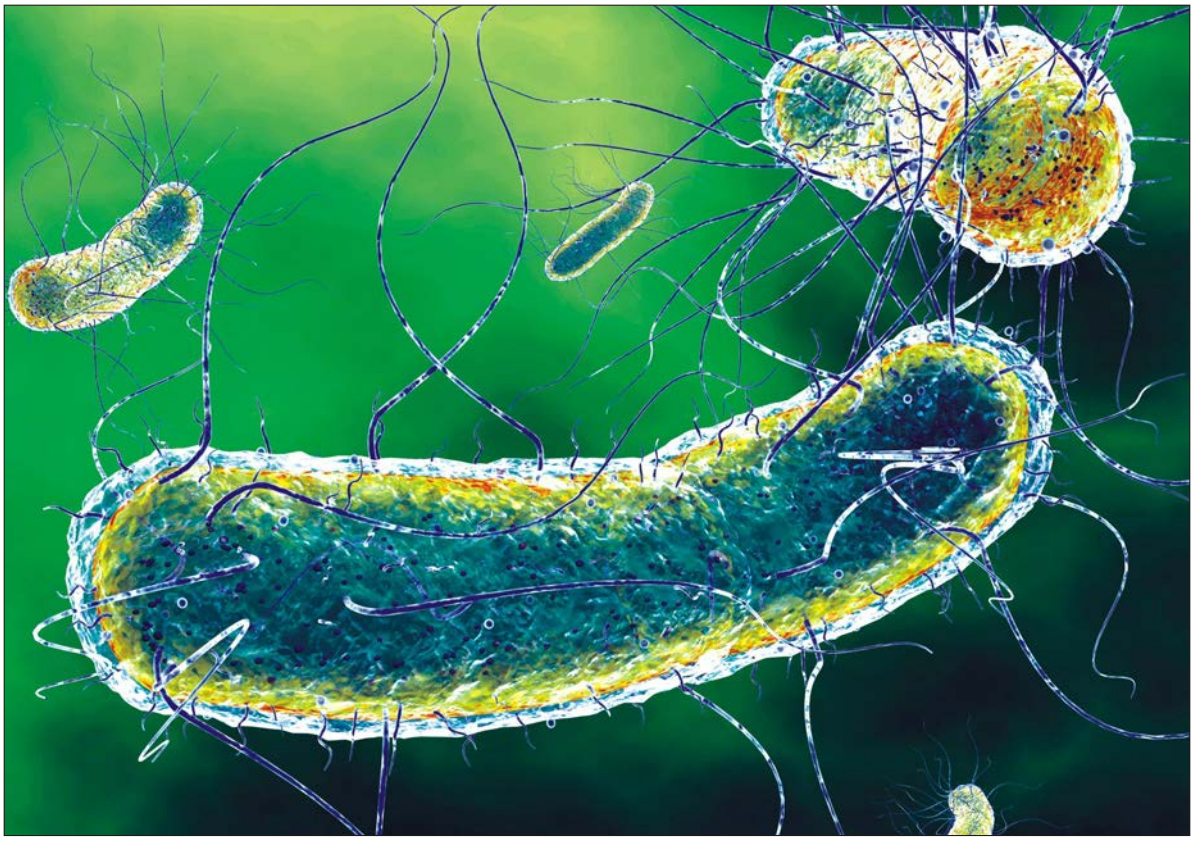

Current Research in Bacteriology

ISSN 1994-5426

science alert

ANSInet
an open access publisher
http://ansinet.com

Current Research in Bacteriology 3 (1): 42-45, 2010
ISSN 1994-5426

In vitro Activity of Ampicillin Against β-Lactamase Producing Enterobacterial Species

[1]A.O. Shakak and [2]H.A. Saeed
[1]Faculty of Medical Laboratory Science, Shendy University, Sudan
[2]College of Medical Laboratory Science,
Sudan University of Science and Technology, Sudan

Abstract: This study was undertaken to determine the *in vitro* activity of ampicillin against β-lactamase producing enterobacterial species. The study was conducted during the period December 2006-August 2009. Two hundred patients were investigated. Clinical specimens collected were 38.5% ear swabs, 36.5% wound swabs and 25% urine specimens. Cultivation of these specimens on enriched and/or differential media yielded 189 bacterial isolates, of which 96 were enterobacterial species. Among the enterobacterial isolates 62.5% were β-lactamase producers as indicated by β-lactamase acidometric method. Ampicillin activity against β-lactamase producers was determined by modified Kirby-Bauer Disc Diffusion technique. The technique was judged and approved by the National Committee for Clinical Laboratory Standards (NCCLS). The results showed that positive β-Lactamase isolates were *E. coli* 21.6%, *K. pneumoniae* 15.5%, *P. mirabilis* 13.7% and *P. vulgaris* 0.9%. The result indicated that only 8.6% of the β-lactamase producers were sensitive to ampicillin while 91.4% were resistant. It has been concluded that further studies are needed to validate the usefulness of ampicillin in treatment of enterobacterial infections in Sudanese patients.

Key words: Ampicillin , β-lactamase, enterobacterial species

INTRODUCTION

The most commonly isolated member of the enterobacteriaceae possess chromosomal group-1 β-lactamases (Bell *et al*., 2003). Extended-Spectrum β-Lactamase (ESBL) producing enterobacteriaceae pose a major health problem as the rate of infection is particularly high (Tandé *et al*., 2009).

Ampicillin a semi-synthetic penicillin, is one of the expanded spectrum penicillins, belonging to the subgroup aminopenicillins. Organisms of medical importance sensitive to ampicillin include *Haemophilus influenzae*, *Escherichia coli*, *Proteus* species, *Salmonella* species and *Shigella* species. These organisms constitute the most important pathogens in the Sudan (Ahmed *et al*., 2000; Saeed *et al*., 2009). Diseases caused by these organisms range from mild infections to life threatening diseases such as meningitis.

On the other hand, Antibacterial preparations containing β-lactams, particularly ampicillin, are widely used for prophylaxis and chemotherapy of avian bacterial infections in Nigeria (Chah and Oboegbulem, 2007). And most of the above mentioned bacteria are β-lactamase producers and have a mechanism to resist the action of ampicillin (Gutmann *et al*., 1988).

Corresponding Author: Dr. Humodi A. Saeed, College of Medical Laboratory Science, Sudan University of Science and Technology, P.O. Box 407, Khartoum, Sudan Tel: +249 183 773074 Fax: 249 183 771512

Ampicillin is an important antibiotic traditionally used to treat meningitis, salmonelosis, shigellosis, *E. coli* infections and *Proteus* infections (Birgul and Nedim, 2007; Bourtchai *et al*., 2008). However, excessive or improper use leads to development of resistant bacteria.

Ampicillin has an action similar to penicillin G. Its differences in antibacterial spectrum compared to penicillin G, can be explained by its greater ability to penetrate the outer membrane of the cell wall of some Gram-negative bacilli.

MATERIALS AND METHODS

The study was undertaken during the period December 2006-August 2009 in a group of hospitals in Khartoum State, including E.N.T. Hospital, Khartoum Teaching Hospital, Omdurman Military Hospital and Alshajara Health Center. Patients suffering from ear, wound and urinary tract infections were included. A total of two hundred specimens were collected.

The patients sampled were aged 1 to 72 years. The specimens from patients with ear and wound infections were collected using sterile cotton swabs, while urine specimens were collected in sterile containers. All specimens were labeled with patients' names and ages.

The specimens were inoculated aseptically on plates of blood agar and MacConkey's agar. All plates were incubated aerobically at 37°C for 16-24 h. The plates were examined for significant growth. The morphological characters of the suspected colonies including sizes, shapes, colors, pigments and hemolysis were studied. Identification of the isolates was done in accordance to Barrow and Feltham (2003). The identified strains were stored for further investigations.

β-Lactamase test rapidly detects the presence of β-lactamase enzyme produced by members of enterobacteriaceae. The enzyme confers resistance to a number of penicillin antibiotics by attaching to a common β-lactam ring structure, resulting in inactivation of the drugs. This mode of action forms the basis of the β-lactamase tests reaction. The test was preformed on ready strip (Whatman No I filter paper impregnated with benzyl penicillin and pH indicator, bromcresol purple). Briefly, the test was done by smearing bacteria under test on the strips after wetting by sterile distilled water. β-lactamase positive organisms produce enzyme, which hydrolyses benzyl penicillin forming penicilloic acid. The later causes a fall in pH, demonstrated by a rapid change in the color of the pH indicator form purple to yellow.

Usually sensitivity tests were performed to assess the activity of ampicillin. The test was done according to Kirby-Bauer method (Vandepitte *et al*., 2002; WHO, 1997), briefly, inocula were prepared by transferring several colonies of the same appearance into 3 mL of normal saline in test tube and adjusting the density of the suspensions to 0.5 McFarland standard.

A plate of Muller-Hinton agar was seeded by immersion of cotton wool swab in the suspension and after removal of excess amount then streaked evenly. The antibiotic discs were placed on the surface of Muller-Hinton agar using forceps. The medium, left for a few minutes at room temperature for diffusion, was then incubated at 37°C overnight.

Zones of inhibition were measured in mm using a ruler. The result was interpreted using interpretative chart and then reported (Cheesebrough, 2000).

A β-lactamase-producing *E. coli* ATCC 25922, was used to control the results of this study.

RESULTS AND DISCUSSION

Primary culture on Blood agar and MacConkey's agar demonstrated bacterial growth in 178 (89%) of the specimens. Mix infection was noted in eleven specimens. All bacterial isolates (189) were identified by their Gram reaction, colonial morphology and conventional biochemical tests. Of the bacterial isolates 96 were Enterobacterial species.

Of the enterobacterial isolates (n = 96), 34.4% were *E. coli*, 27.0% were *P. mirabilis*, 33.3% were *K. pneumoniae* and 5.3% were *P. vulgaris* (Table 1). Biochemical properties of these isolates were similar to those described by Cruickshank *et al.* (1975) and Cheesebrough (2000). The majority of *E. coli* isolates (78.8%) were recovered from urine, the rest were recovered from wound and ear swabs (15.1 and 6.1%), respectively. *Proteus mirabilis* (n = 32) were recovered from wounds (37.5%), urine and ear swabs (31.25% each). *Klebsiella pneumoniae* (n = 26) were recovered from wounds (42.3%), ear swabs (38.5%) and urine (19.2%). *Proteus vulgaris* (n = 5) were recovered from urine and ear swabs (40% each) and wounds (20 %) (Table 1).

The enterobacterial isolates were subjected to Acidometric method. The results showed 62% isolates, were β-lactamase producers. Among these, there were 25 *E. coli*, 18 *K. pneumoniae*, 16 *P. mirabilis* and 1 *P. vulgaris* (Table 2).

Results displayed in Table 2 revealed that 21.6% isolates of *E. coli*, 15.5% isolates of *K. pneumoniae,* 13.7% isolates of *P. mirabilis* and 21.9% isolates of *P. vulgaris* were β-lactamase producers. The results on dominance of β-lactamase producers among *E. coli* and *K. pneumoniae* are consistent with those reported by Watanabe *et al.* (2002). Among β-lactamase producers (Table 3), 100% of *E. coli*, 94.4% of *K. pneumoniae*, 87.5% of *P. mirabilis* and 100% of *P. vulgaris* were ampicillin resistant. These results indicate dominance of ampicillin resistance among β-lactamase producers.

Table 1: Pathogenic organisms isolated from clinical specimens

	Clinical specimens			
Organism isolated	E	W	U	Total
Escherichia coli	2	5	26	33
Klebsiella pneumoniae	10	11	5	26
Proteus mirabilis	10	12	10	32
Proteus vulgaris	2	1	2	5
Total	24	29	43	96

E: Ear, W: Wound, U: Urine

Table 2: β-lactamase producers among isolates

	β-lactamase		
Organism isolated	+ ve	-ve	Total
Escherichia coli	25	8	33
Klebsiella pneumoniae	18	8	26
Proteus mirabilis	16	16	32
Proteus vulgaris	1	4	5
Total	60	36	96

Table 3: Sensitivity results of the β-lactamase producers to ampicillin

		No. of sensitive and resistant organisms	
Organism isolated	No.	Sensitive	Resistant
Escherichia coli	25	0	25
Klebsiella pneumoniae	18	1	17
Proteus mirabilis	16	2	14
Proteus vulgaris	1	0	1
Total	60	3	57

It is noteworthy that a significant proportion ($p<0.05$) of the isolates (8.6%) (Table 3) were β-lactamase producers, but were susceptible to ampicillin. This finding necessitates further investigations. Based on dominance of ampicillin resistance enterobacterial isolates, it is concluded that further studies are needed to validate the usefulness of ampicillin in treatment of enterobacterial infections in Sudanese patients.

REFERENCES

Ahmed, A.A., H. Osman, A.M. Musa, H.A. Mansour, A.B. Ahmed and Z.Z. Karrar, 2000. Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. Am. J. Trop. Med. Hyg., 63: 259-263.

Barrow, G.I. and R.K.A. Feltham, 2003. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edn., Cambridge University Press, Cambridge, pp: 352.

Bell, J.M., J.D. Turnidge and R.N. Jones, 2003. Prevalence of extended-spectrum-lactamase-producing enterobacter cloacae in the Asia-pacific region results from the SENTRY antimicrobial surveillance program, 1998 to 2001. Antimicrob Agent Chemother, 47: 3989-3993.

Birgul, K. and S. Nedim, 2007. *In vitro* susceptibilities of escherichia coli and klebsiella spp to ampicillin-sulbactam and amoxicillin-clavulanic acid. Jap. J. Infect. Dis., 60: 227-229.

Bourtchai, L.B., P.M. Kurkdjian, E. Bingen, I. Filliol and A. Dhalluin *et al*., 2008. Macrolide-Resistant *Shigella sonnei*. Emerging Infec. Dis., 14: 1297-1299.

Chah, K.F. and S.I. Oboegbulem, 2007. Extended-spectrum beta-lactamase production among ampicillin-resistant Escherichia coli strains from chicken in Enugu State, Nigeria. Braz. J. Microbiol., 38: 681-686.

Cheesebrough, M., 2000. District Laboratory Practice in Tropical Countries. 1st Edn., Press Syndicate of the University of Cambridge, Cambridge UK., pp: 132-143.

Cruickshank, R., J.P. Duguid, B.P. Mariom and R.H.A. Swain, 1975. Medical Microbiology the Practice of Medical Microbiology. 12th Edn., Livingsone, London.

Gutmann, L., R. Williamson, E. Collatz and J.F. Acar, 1988. Mechanisms of Beta-lactam Resistance in *Haemophilus influenzae*. Eur. J. Clin. Microbiol. Infect. Dis., 7: 610-615.

Saeed, H.A., Z.K. Yousif, M.M. El-Hassan, M.E.A. Atti and M.M. Mansour, 2009. A real-time polymerase chain reaction based assay for the detection of escherichia coli in patients with urinary tract infection in the Sudan. Res. J. Microbiol., 4: 173-177.

Tandé, D., N. Jallot, F. Bougoudogo, T. Montagnon, S. Gouriou and J. Sizun, 2009. Extended-spectrum β-lactamase–producing enterobacteriaceae in Malian Orphanage. Emerging Infec. Dis., 15: 472-474.

Vandepitte, J., J. Verhaegen, K. Engbaek, P. Rohner, P. Piot and C.C. Heuck, 2002. Basic Laboratory Procedures in Clinical Bacteriology. 7th Edn., World Health Organization, Geneva, ISBN: 9241544252.

Watanabe, A., S. Yasui, Y. Murayama, Y. Tokue and H. Takahashi *et al*., 2002. Antibiotic susceptibility and beta-lactamase activity of pathogenic microbes isolated in Miyagi Prefecture between December in 1999 and February in 2000. Jap. J. Antibiot., 55: 42-53.

WHO, 1997. Manual on Antimicrobial Resistance and Susceptibility Testing. WHO, UK., pp: 62-65.